Integrated Leafy Spurge (*Euphorbia esula*) Control Using Imazapic, *Aphthona* spp. Biological Control Agents, and Seeded Native Grasses

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Chemical, cultural, and biological methods have been developed to control leafy spurge in a variety of environments. *Aphthona* spp. biological control agents have established throughout the northern Great Plains and Rocky Mountain region and successfully controlled leafy spurge in many areas, but notable exceptions include areas with sandy soils. Leafy spurge control can be improved by combining methods such as chemical, biological, or cultural treatments, compared to a single method used alone. The effects of *Aphthona* spp., imazapic herbicide, and interseeded native grass species alone or in combination for leafy spurge control were evaluated at two locations in southeastern North Dakota for 5 yr. Both the Sheyenne National Grassland (SNG) and Walcott, ND, study locations had greater than 80% sand soil. Leafy spurge stem density, canopy cover, and yield were reduced for 1 to 2 yr in all treatments that included imazapic, with no difference in control between single and combination treatments. *Aphthona* spp. and interseeded native grasses alone or combined did not reduce leafy spurge density or cover. *Aphthona* spp. population remained low throughout the study at both locations. Forb yield increased during the study at the SNG but not the Walcott location. Conversely, warm-season grass yield increased at Walcott but not at the SNG. Leafy spurge stem density declined from 92 to 50 stems/m^2 in 5 yr at the SNG site. The decline could not be attributed to specific treatments applied in this study and may be due to self-limitation or soil pathogens.

Nomenclature: Imazapic; leafy spurge, *Euphorbia esula* L. EPHES.

Key words: Biological weed control, integrated pest management, invasive weed, interseeding, flea beetle.

Leafy spurge (*Euphorbia esula* L.) is native to Eurasia and is an invasive perennial weed generally found in untilled lands across the Great Plains of North America (Dunn 1979). The weed can reduce most native flora and fauna, with plant diversity decreased by over 80% in only a few years (Messersmith and Lym 1983; Selleck et al. 1962). Chemical, biological, and cultural methods have been used to control leafy spurge (Lym 1998). Herbicides have been the most common control method and include picloram plus 2,4-D, dicamba, quinclorac, and imazapic. Biological control agents, such as the introduced flea beetles *Aphthona nigriscutis* Foudras, *Aphthona czwalinae* Weise, and *Aphthona lacertosa* Rosenheim, are examples of insects successfully introduced to control leafy spurge (Hodur et al. 2006b). Interseeded native grasses, such as Indiangrass [*Sorghastrum nutans* (L.) Nash], western wheatgrass [*Pascopyrum smithii* (Rydb.) A. Löve], and big bluestem (*Andropogon gerardii* Vitman), have also reduced leafy spurge through competition (Lym and Tober 1997; Masters and Nissen 1998). Although leafy spurge is not utilized by cattle, it is readily grazed by sheep and goats, which provides short-term top-growth reduction (Landgraf et al. 1984).

Leafy spurge biological control with insects is the most cost-effective and likely the best long-term control method (Bangsund et al. 1999; Butler et al. 2006; Hodur et al. 2006a; Kirby et al. 2000). Although *Aphthona* spp. have become established at locations throughout the region, the success rate in some areas is very low. Flea beetles released where leafy

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Interpretive Summary

Imazapic reduced leafy spurge stem density, foliar canopy cover, and biomass for 1 to 2 YAT, but leafy spurge reestablished by 3 and 4 YAT in the sandy soils of this study. Neither the *Aphthona* spp. biological control agents nor interseeded native grass treatments alone or in combination reduced the leafy spurge infestation. Although imazapic reduced the infestation initially, long-term control will require periodic retreatments, which are often cost-prohibitive for large infestations. Long-term herbicide use will reduce native species diversity, especially of forb species, and can cause environmental problems such as ground-water contamination, particularly in areas with sandy soils and high water tables.

Cultural control methods such as grazing with sheep or goats are options for controlling leafy spurge infestations in areas of sandy soil. Grazing has been combined with herbicides for increased long-term leafy spurge control and could be implemented to reduce leafy spurge root density and seed production. Grazing with goats on the SNG is a current practice and one that will likely continue for the foreseeable future. Unfortunately, leafy spurge control in sandy soils will likely remain problematic with annual grazing and periodic use of herbicides the most reliable options until and unless a biological control agent (insect or pathogen) more adapted to this environment is found.

spurge is growing in sandy (> 80%) soil have less probability of insect establishment and subsequent weed control (Lym 2005). For example, *A. nigriscutis*, *A. czwalinae*, and *A. lacertosa* were released at two North Dakota State University research locations 30 km (18.6 mi) apart on the same date. All *Aphthona* species established and controlled leafy spurge at one location but never increased in population enough to be effective at the other (R. G. Lym, unpublished data). The major difference between the two locations was the amount of sand in the soil; insects established in soil with 45% sand but did not establish in soil that averaged 85% sand.

The poor establishment of *Aphthona* spp. on leafy spurge growing in sandy soil may be because of the root system. Leafy spurge fine root structure is commonly found deeper in sandy than loamy soils, which may prevent the newly hatched larvae from finding a food source quickly enough to survive (Mundal and Carlson, 1999). Newly hatched *Aphthona* spp. larvae require a nearby food source of filamentous roots close to the soil surface (Lym and Nelson 2002; Mundal and Carlson 1999). Leafy spurge growing in extremely sandy soils produces fewer filamentous or lateral roots close to the soil surface, which reduces the food source for the first instars, thereby limiting survival. Leafy spurge grows very well in sandy soils throughout the Northern Great Plains. If biological control agents cannot be established in these areas, then leafy spurge will continue to expand and other methods, such as treatment with herbicides, will likely be needed indefinitely.

Herbicides have been successfully combined with *Aphthona* spp. resulting in both increased biological control agent population and subsequent leafy spurge control (Lym 2005; Lym and Nelson 2002; Nelson and Lym 2003). For example, leafy spurge was reduced from 82 stems/m^2 before treatment to 12 stems/m^2 during the next growing season after treatment with picloram plus 2,4-D at 0.56 plus 1.1 kg/ha (0.5 plus 1 lb/ac) fall-applied to leafy spurge infested with *A. nigriscutis* (Lym 1998). The leafy spurge density gradually declined when only insects were present, but they took 3 yr to reduce the infestation to the same level achieved in 1 yr by the herbicide-plus-insect combination treatment.

Leafy spurge control programs that include establishment of introduced and native perennial grasses (both warm- and cool-season) have resulted in increased herbage production and improved long-term weed control compared to single-method programs (Ferrell et al. 1998; Lym and Tober 1997; Masters and Nissen 1998). However, incorporation of biological control agents with reseeding has been difficult, primarily because of the cultural methods, such as cultivation and top-growth control, of all plant material required to establish a productive stand of competitive species. Seeding of competitive species using a no-till planter to avoid cultivation would be less detrimental to an established leafy spurge biological control agent than conventional seeding techniques. Unfortunately, no-till seeding of desirable species has not lead to successful stand establishment to date except when the site was mowed or burned prior to seeding and an herbicide was applied to control invasive cool-season grasses such as Kentucky bluegrass (*Poa pratensis* L.) (Masters and Nissen 1998; Masters et al. 2001).

A combination of biological control agents with competitive grasses, herbicides, or both could provide better leafy spurge control than any single method used alone, especially in areas where the agent has established but the population has remained too low to reduce weed density. Several years following a study to control leafy spurge using competitive grasses in an area with fine loamy over sandy soil (Lym and Tober 1997), *Aphthona* spp. were observed to establish and increase in population. Intentional releases of the biological control agents had not previously established and *Aphthona* spp. had moved into this area unaided. Perhaps the competition from the seeded grasses had forced leafy spurge to establish fine feeder roots closer to the surface than normally found in sandy soil. The objectives of this research were to determine the effects of imazapic, *Aphthona* spp. biological control agents, and native grass species in combination for control of leafy spurge in areas with sandy soils and a history of poor *Aphthona* spp. establishment.

Materials and Methods

Leafy spurge control using imazapic, *Aphthona* spp. flea beetles, and interseeded native grasses alone or combined was evaluated in a field experiment conducted at two locations: the SNG (T135N, R54W, Section 26; 46.28°N, 97.20°W), established in 2001, and a location near

Table 1. Annual precipitation for 2000 through 2006 at the Sheyenne National Grassland and Walcott research locations in southeastern North Dakota.

	Sheyenne National Grassland		Walcott	
Year	Actual[a]	Departure[b]	Actual[a]	Departure[b]
	cm			
2000	57.3	+5.1	81.6	+29.4
2001	58.6	+6.4	58.0	+5.8
2002	39.7	−12.5	48.7	−3.5
2003	48.5	−3.7	45.4	−6.8
2004	67.6	+15.4	65.4	+13.2
2005	81.9	+29.7	66.7	+14.5
2006	69.7	+17.5	66.8	+14.6

[a] Data obtained from National Oceanic and Atmospheric Administration weather station at McLeod, ND, approximately 29 km west of the Sheyenne National Grassland site and at Chaffee, ND, approximately 45 km northwest of the Walcott site.

[b] Departure from 30-yr average for 1971 through 2000.

Walcott, ND, on the Albert Ekre Research Center of North Dakota State University (T135N, R 51W, Section 6; 46.33°N, 97.08°W), established in 2002. Both locations consisted of well-drained soils with greater than 80% sand and in both locations *Aphthona* spp. had failed to establish in high enough populations to effect leafy spurge control. The SNG location had a Hecla-Maddock soil (sandy, mixed, frigid, Oxyaquic Hapludolls-sandy, mixed, frigid Entic Hapludolls), and the Walcott location a Serden soil (mixed, frigid, Typic Upidsamments). Annual precipitation at both locations exceeded the 30-yr average of 52.2 cm (20.6 in) in 2000, 2001, 2004, and 2005; but was below average in 2002, 2003, and 2006 (Table 1).

The major plant communities of both locations included sedge meadow, tallgrass prairie, and mixed-grass prairie (Seiler 1973). Both locations are remnants of the tallgrass prairie and had been subject to severe overgrazing. The predominant vegetation of the SNG location included the nonnative species Kentucky bluegrass and leafy spurge, with a variety of native grasses including blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths], big bluestem, and little bluestem [*Schizachyrium scoparius* (Michx.) Nash-Gould]. The Walcott location primarily consisted of Kentucky bluegrass and leafy spurge, with nonnative smooth bromegrass (*Bromus inermis* Leyss.) scattered throughout at less than 5% of the total cover.

The experiment was a randomized complete block design with a split-block two (± *Aphthona*) by three (imazapic, warm-season grass, or cool- plus warm-season grass) factorial arrangement and four replicates at each site (Table 2). Whole-plot treatments included imazapic alone, interseeded warm- plus cool-season grasses alone or combined with imazapic, interseeded warm-season grasses alone or combined with imazapic, and an untreated control. Subplots were segregated by insect and noninsect treatments. Whole plots varied slightly in size based on the leafy spurge infestation. At the SNG location plots were 3.7 by 12.2 m (12 by 40 ft), divided into two subplots of 3.7 by 6.1 m and at the Walcott location were 4.6 by 12.2 m, divided into two subplots of 4.6 by 6.1 m.

Biological control was initiated the first year of the study at each location (Table 2). A total of 500 *Aphthona czwalinae/lacertosa* flea beetles were released in each insect subplot in late June of 2001 for the SNG and 2002 for the Walcott locations. Noninsect subplots were treated annually with the systemic insecticide imidacloprid [1-((6-chloro-3-pyridinyl) methyl)-*N*-nitro-2-imidazolidinimine] at 1.4 kg ai/ha (1.25 lb/ac) in May prior to *Aphthona* emergence and again 6 wk later if necessary. *Aphthona* spp. overwinter in the larval stage then pupate and emerge as adults in late spring or early summer. The adult female then mates and lays eggs on the soil surface for approximately 3 wk (Gassmann et al. 1996). The insecticide was present in the root system during this time and prevented the newly hatched *Aphthona* larvae from establishing on leafy spurge roots in the noninsect plots. Larvae were never found on leafy spurge roots in soil cores

Table 2. Treatments and application sequence for leafy spurge control in an integrated management program evaluated for 5 yr at two sites in North Dakota.

Whole-plot treatment	Application year	Subplot treatment[a]
Control	1	± *Aphthona* spp.
Imazapic	2	± *Aphthona* spp.
Cool- and warm-season native grass species	3	± *Aphthona* spp.
Imazapic[b] + cool- and warm-season native grass species	2 then 3	± *Aphthona* spp.
Warm-season native grass species	3	± *Aphthona* spp.
Imazapic[b] + warm-season native grass species	2 then 3	± *Aphthona* spp.

[a] *Aphthona* spp. released in all of the insect subplots during June of the first year of the study.

[b] Imazapic was applied in September of the second year of the study, then grasses were seeded the following May (third year).

Table 3. Two native grass mixtures of warm-season grass species and warm- plus cool-season grass species interseeded in the third year of the integrated pest management study at the Sheyenne National Grassland and Walcott research locations in southeastern North Dakota.

Native grass mixtures	Mix	Pure live seed
	%	kg/ha
Warm- plus cool-season grass species		
Pascopyrum smithii (Rydb.) A. Löve, cv. Rodan 'Rodan' western wheatgrass	10	1.7
Nassella viridula (Trin.) Barkworth, cv. Lodorm 'Lodorm' green needlegrass	25	3.1
Elymus canadensis L., cv. Mandan 'Mandan' Canada wildrye	15	1.9
Andropogon gerardii Vitman, cv. Bison 'Bison' big bluestem	25	3.1
Panicum virgatum L., cv. Dacotah 'Dacotah' switchgrass	25	1.9
Warm-season grass species		
Bouteloua curtipendula (Michx.) Torr., var. *curtipendula*, cv. Pierre 'Pierre' sideoats grama	15	1.9
Bouteloua gracilis (Willd. ex. Kunth) Lag. ex. Griffiths, cv. Bad River 'Bad River' blue grama	15	0.7
Andropogon gerardii Vitman, cv. Bison 'Bison' big bluestem	25	3.1
Sorghastrum nutans (L.) Nash, cv. Tomahawk 'Tomahawk' indiangrass	25	2.9
Panicum virgatum L., cv. Dacotah 'Dacotah' switchgrass	20	1.6

taken each fall from these plots. There were 2-m buffer strips between the insect and noninsect plots, which prevented imidacloprid translocation into insect-treated plots.

The *Aphthona* spp. population was evaluated each year at both locations in mid-June during peak *Aphthona* emergence. Population estimates were based on counts of flea beetles per five sweeps using a standard insect sweep 36-cm-diam (14-in-diam) sweep net. Three 1-m^2 areas (front, middle, and back portions of each plot) were swept in each plot. Insects were released back into the same plot immediately after counting.

Imazapic at 105 g ai/ha (1.5 oz/ac) plus a methylated seed oil (MSO)[1] at 2.3 L/ha (1 qt/ac) was fall-applied in mid-September of the second year of the study to appropriate plots, about 15 mo after the *Aphthona* spp. flea beetle release (Table 1). All imazapic treatments were applied with a handheld boom sprayer pressurized by CO_2 at 240 kPa (35 lb/sq. in) and calibrated to deliver 160 L/ha (17 gal/ac) using four 8002 flat-fan nozzles.[2] Both imazapic and picloram provide good leafy spurge control when fall-applied, but picloram could not be used in this study because of a high water table (< 3 m) at the SNG location.

The two native grass treatments were interseeded into standing vegetation in May of the third year at each site, which was approximately 2 yr after *Aphthona* spp. were released and 9 mo after imazapic application (Table 2). Two mixtures of grasses, which included warm-season grass species and warm- plus cool-season grass species, were interseeded using a Truax flexicoil® seeder[3] (Table 3). The species and seeding rates were those recommended by the U.S. Department of Agriculture Natural Resources Conservation Service technical guide standards (Sedivec et al. 2001).

Leafy spurge stem density was determined in early June prior to the first treatment and annually thereafter by counting the number of stems in four permanent quadrats (50 by 50 cm) centrally located down the long axis of each subplot when leafy spurge was in the flowering growth stage. Leafy spurge seedlings were not included in the density estimation because seedlings have approximately 82% mortality (Hanson and Rudd 1933; Selleck et al. 1962) and were identified based on the presence of cotyledons and height of 4 to 6 cm. Percentage of foliar cover of leafy spurge, grasses, forbs, shrubs, bare ground, and litter was determined utilizing five quadrats (20 by 50 cm) sampled centrally down the long axis of each subplot (Daubenmire 1959; Stohlgren et al. 1998).

Vegetation was harvested in August at peak standing biomass both prior to herbicide treatment and every year thereafter to determine herbage production. Vegetation was clipped in four quadrats (0.25 by 0.25 m) per subplot at a plant height of 8 cm to avoid killing any interseeded native grass seedlings. Samples were hand-separated into the categories of leafy spurge, *Poa* spp., warm-season grasses, other cool-season grasses, forbs, and shrubs. The vegetation samples from each quadrat were placed in individual bags, dried at 45 C (113 F) for 7 d, and weighed to determine herbage production and leafy spurge biomass. Vegetation was mowed in mid-October and raked in the spring for the first 2 yr of the study in all plots, which aided in native grass establishment by removing accumulated thatch.

Kentucky bluegrass invaded a portion of the first replicate at the Walcott location following the imazapic treatment. Glyphosate at 680 g ae/ha was applied in late April 2004 to reduce Kentucky bluegrass competition in the first replicate and enhance interseeded grass establishment in the compet-

Table 4. Change in leafy spurge stem density over time as affected by *Aphthona* spp. flea beetles, imazapic, and interseeded native grass species at the Sheyenne National Grassland and Walcott, ND, locations.[a]

	Sheyenne National Grassland					Walcott				
Treatment	2001	2002	2003	2004	2005	2002	2003	2004	2005	2006
	stems/m^2									
Aphthona spp.[b]	84	84	48	42	30	48	44	27	52	44
Without *Aphthona* spp.	100	104	55	67	47	48	36	21	55	48
LSD (0.05)	NS	NS	NS	23	NS	NS	NS	NS	NS	NS
Imazapic[c]			7	30	26			1	47	44
Without imazapic			94	79	51			47	60	49
LSD (0.05)			37	46	NS			19	12	NS
Warm-season grasses[d]				40	32				62	58
Warm and cool mixture				47	30				57	43
Without grasses				76	53				42	37
LSD (0.05)				NS	NS				NS	19

[a] There was no interaction among treatments so main effects were averaged over all two- and three-way treatments.

[b] *Aphthona* spp. flea beetles were released in June 2001 at the Sheyenne National Grassland location and in June 2002 at the Walcott location.

[c] Imazapic at 105 g/ha was applied in September 2002 at the Sheyenne National Grassland location and September 2003 at the Walcott location.

[d] Native grass species were interseeded in May 2003 at the Sheyenne National Grassland location and May 2004 at the Walcott location.

itive grass plots. Glyphosate treatment was not necessary at the SNG location.

The data were analyzed using an ANOVA. A Bartlett's chi-square test ($P \leq 0.001$) was performed to determine homogeneity of error mean squares from each experiment, but the yearly data were not homogeneous between locations so a combined ANOVA was not conducted. There was no interaction between single and multiple treatments for biomass or cover at either location. Thus, data for single treatments were averaged over two- and three-way treatments (factorials). Transformation of percentage of foliar cover data to arcsine square root did not change interpretation of results. Therefore, untransformed data are presented. Treatment means were separated using Fischer's Protected LSD and linear contrasts, which were considered significant at $P < 0.05$.

Results and Discussion

There was no difference in control between the single and combination treatments of biological control agents, interseeded native grasses, or both during the 5-yr period of this study (Table 4). *Aphthona* spp. did not reduce leafy spurge stem density at either location, except in 2004 at the SNG (37% control). Leafy spurge was reduced for 2 yr or less only with treatments that included the herbicide imazapic. Prior to treatment, leafy spurge density at the SNG averaged 92 stems/m^2 and at Walcott, 48 stems/m^2. Leafy spurge density 1 yr after treatment (YAT) with imazapic was reduced from 94 to 7 stems/m^2 (92% control) in 2003 at the SNG location and from 47 to 1 stem/m^2 (98% control) in 2004 at Walcott when averaged over the two- and three-way combination treatments. Stem density reduction 2 YAT with imazapic averaged 62% at the SNG and 22% at Walcott. In general, leafy spurge stem density reduction 1 YAT with imazapic at 105 g/ha was greater in this study (91% and 98% control) compared to previous research with imazapic at 140 g/ha which averaged 72% control (Markle and Lym 2001). The increase in control was likely due to the herbicide moving deeper in the soil profile in the very sandy soil of these research sites compared to the loamy soils in the previous study.

Leafy spurge stem density was not reduced by either interseeded grass treatment at either location and actually increased in the warm-season grass treatment at Walcott by the end of the study (Table 4). Stem density in the interseeded warm-season grass treatment at Walcott was 58 stems/m^2 compared to 43 stems/m^2 in the interseeded warm- plus cool-season grass treatment and only 37 stems/m^2 in treatments without interseeded grasses.

The integrated treatment method used in this study did not reduce leafy spurge stem density (Table 4) even though

combinations of *Aphthona* spp. and herbicides, such as imazapic or picloram plus 2,4-D, have successfully reduced leafy spurge compared to herbicides or *Aphthnoa* spp. used alone in previous studies (Lym 2005; Lym and Nelson 2002). The failure of *Aphthona* spp. to establish a high population likely contributed to the lack of long-term reduction in leafy spurge.

The *Aphthona* spp. population at the SNG and Walcott remained low (less than 25 beetles/m^2) throughout the study (data not shown) compared to locations where *Aphthona* spp. have reached several hundred beetles per square meter and controlled leafy spurge (Lym and Nelson 2000; Hodur et al. 2006a and b). The low *Aphthona* spp. populations at both locations are probably a result of the sandy soil texture. Leafy spurge fine roots grow too deep in sandy soil for newly hatched larvae to reach and begin feeding in time to survive (Lym and Nelson 2002; Mundal and Carlson 1999). Although previous observations indicated an increase in *Aphthona* spp. population following native grass seeding in sandy soil (R. G. Lym, unpublished data), no increase occurred in this study.

In general, there was little change in canopy cover by desirable or weedy species at either study location (Table 5). Leafy spurge canopy cover was not affected by *Aphthona* spp. at either location. Leafy spurge canopy cover at the SNG was reduced by imazapic in 2003 (1 YAT) from 25 to 5% and in 2004 (2 YAT) from 6 to 3% when averaged over the two- and three-way combination treatments. Grass cover following imazapic treatment at the SNG increased initially in 2003 but was not different among treatments by the end of the study.

Leafy spurge cover at Walcott was also reduced with imazapic in 2004 (1 YAT) from 24 to 10% whereas grass cover increased 1 YAT from 25 to 42% (Table 5). However, leafy spurge cover was similar regardless of treatment by the end of the study, and averaged 19% in 2006. Similar to the SNG, there was only a 1-yr increase in grass cover (2004).

Leafy spurge biomass was reduced by imazapic 1 and 2 YAT at the SNG and Walcott, but was unaffected 3 and 4 YAT (Tables 6 and 7). Leafy spurge biomass following imazapic treatment at the SNG was reduced in 1 and 2 YAT from 268 to 40 kg/ha, an 85% reduction, and 216 to 76 kg/ha a 65% reduction, respectively, when averaged over the two- and three-way combination treatments (Table 6). Leafy spurge biomass at Walcott was reduced 1 YAT from 612 to 80 kg/ha (87% reduction) and 2 YAT from 946 to 484 kg/ha (49% reduction) (Table 7). The decrease in leafy spurge biomass at Walcott 1 YAT (2004) with imazapic resulted in a 34% reduction in total herbaceous biomass (1,420 to 932 kg/ha).

Aphthona spp. and interseeded grass treatments did not reduce leafy spurge biomass at either study location, regardless if used alone or combined with other treatments (Tables 6 and 7). Initially *Poa* spp. yields increased following the imazapic treatment at SNG (Table 6). Also, forb yield in 2005 (3 YAT) increased 46% with imazapic (584 to 848 kg/ha) and increased 59% with the interseeded warm-season grass treatment (540 to 860 kg/ha).

Unlike at the SNG, *Poa* spp. yield at Walcott decreased following imazapic and interseeded grass treatments, while warm-season grass production increased (Table 7). *Poa* spp. production at Walcott was reduced 48% in 2005 (2 YAT) from 396 to 204 kg/ha with imazapic. *Poa* spp. yield was also reduced 81% (628 to 116 kg/ha) with the interseeded warm-season grass treatment and 75% with the interseeded warm- plus cool-season grass treatment.

Warm-season grass production increased at Walcott 1 YAT with imazapic from 64 to 288 kg/ha and 2 YAT from 60 to 232 kg/ha (Table 7). Warm-season grass yield also increased in 2005 from 20 to 296 kg/ha, nearly a 15-fold increase with the interseeded warm- plus cool-season grass treatment at Walcott, which was not observed at the SNG (Tables 6 and 7). The increased yield continued until the end of the study and averaged 112 kg/ha compared to only 13 kg/ha in nonseeded plots. However, unlike the SNG, forb yield was unchanged by any treatment.

Although combining various treatment methods has helped to control leafy spurge in previous studies, that methodology did not work here. Imazapic was the only treatment that provided a short-term reduction in leafy spurge and control was similar if the herbicide was used alone or in combination with biocontrol agents and/or seeded grasses.

Despite overall inadequate leafy spurge control, there was a general trend for stem density to decline during the 5-yr study at the SNG from an average of 92 stems/m^2 in 2001 to 50 stems/m^2 in 2005 that could not be attributed to any specific treatment or treatment combination (Table 4). A study by Larson and Grace (2004) found that dense patches of leafy spurge experience self-limitation and the denser the patch, the more likely the infestation will decline in subsequent years. The observed decline in stem density was unrelated to the effect of *Aphthona* flea beetles present on the leafy spurge. The results of this study support those findings as the leafy spurge stand declined over time at the SNG site, but not at the Walcott location where the initial leafy spurge stem count was only 52% the density of the SNG location.

Although leafy spurge is found as part of the plant community in its native range of Europe and Russia, the plant seldom forms dense patches such as those found in the northern Great Plains and is relatively rare except for roadside and waste areas (Masters et al. 1992). Besides the presence of many natural insect enemies, leafy spurge in Europe is also attacked by disease caused by soil-borne pathogens (Caesar et al. 1998). A combination of flea

Table 5. Change in foliar canopy cover over time as affected by *Aphthona* spp., imazapic, and interseeded native grasses at the Sheyenne National Grassland and Walcott, ND, locations.[a]

Treatment[b]	Sheyenne National Grassland			Walcott		
	Leafy spurge	Grass	Forbs	Leafy spurge	Grass	Forbs
	%					
	2002			2003		
Aphthona spp.	29	20	11	23	50	1
Without *Aphthona* spp.	31	18	13	19	45	3
LSD (0.05)	NS	NS	NS	NS	NS	1
	2003			2004		
Aphthona spp.	17	34	17	20	33	3
Without *Aphthona* spp.	13	38	15	14	34	6
LSD (0.05)	NS	NS	NS	NS	NS	NS
Imazapic[c]	5	44	15	10	42	3
Without imazapic	25	29	17	24	25	6
LSD (0.05)	10	9	NS	6	9	NS
	2004			2005		
Aphthona spp.	4	36	13	27	45	6
Without *Aphthona* spp.	5	35	12	29	40	10
LSD (0.05)	NS	NS	NS	NS	NS	3
Imazapic	3	38	15	24	47	9
Without imazapic	6	33	9	33	38	8
LSD (0.05)	3	4	5	NS	NS	NS
Warm-season grasses[d]	6	34	14	27	41	10
Warm- plus cool-season grasses	5	34	12	26	38	11
Without grasses	4	38	10	31	49	4
LSD (0.05)	NS	NS	NS	NS	NS	NS
	2005			2006		
Aphthona spp.	15	37	25	19	29	5
Without *Aphthona* spp.	17	38	25	19	23	7
LSD (0.05)	NS	NS	NS	NS	NS	NS
Imazapic	12	38	27	17	23	8
Without imazapic	20	35	23	20	29	4
LSD (0.05)	NS	NS	NS	NS	NS	NS
Warm-season grasses[d]	14	34	29	18	27	6
Warm- plus cool-season grasses	16	38	26	20	22	7
Without grasses	19	38	20	19	30	5
LSD (0.05)	NS	NS	NS	NS	NS	NS

[a] Yearly results were reported for the established treatments only. There was no interaction among treatments so main effects were averaged over all two- and three-way treatments. Data for bare ground and litter not shown.

[b] *Aphthona* spp. flea beetles were released in June 2001 (Sheyenne National Grassland) and 2002 (Walcott).

[c] Imazapic at 105 g/ha was applied in September 2002 (Sheyenne National Grassland) and 2003 (Walcott).

[d] Native grasses were interseeded in May 2003 (Sheyenne National Grassland) and 2004 (Walcott).

beetles with *Fusarium oxysporum* or *Rhizoctonia solani* or both fungi resulted in greater leafy spurge injury than any of the agents used alone (Caesar 2003). Caesar has also suggested that as leafy spurge stem density increases, soil pathogens increase and help to limit the spread and or reduce leafy spurge infestations (Caesar 1996, 2003).

To date, insect biological control agents have done little to control leafy spurge in sandy soils and long-term herbicide

Table 6. Change in herbage biomass as affected by *Aphthona* spp. flea beetles, imazapic, and interseeded native grasses at the Sheyenne National Grassland, ND[a].

Year and treatment	Leafy spurge	*Poa* spp.	Other grass[b]	Forbs	Cool-season grasses	Warm-season grasses	Total[c]
	dry weight (kg/ha)						
2002							
Aphthona spp.[d]	427	412	468	62	–	–	1,382
Without *Aphthona* spp.	395	523	295	54	–	–	1,337
LSD (0.05)	NS	96	NS	NS	–	–	NS
2003							
Aphthona spp.	132	268	200	236	–	–	888
Without *Aphthona* spp.	176	316	152	216	–	–	916
LSD (0.05)	NS	NS	NS	NS	–	–	NS
Imazapic[e]	40	336	156	212	–	–	792
Without imazapic	268	252	196	236	–	–	1,016
LSD (0.05)	188	80	NS	NS	–	–	NS
2004							
Aphthona spp.	100	332	–	408	176	152	1,168
Without *Aphthona* spp.	192	384	–	280	136	184	1,176
LSD (0.05)	NS	NS	–	NS	NS	NS	NS
Imazapic	76	436	–	436	168	204	1,320
Without imazapic	216	280	–	248	148	132	1,024
LSD (0.05)	140	152	–	NS	NS	NS	NS
Warm-season grasses[f]	172	140	–	428	368	188	1,296
Warm- plus cool-season grasses	128	188	–	382	388	188	1,274
Without grasses	140	144	–	212	316	128	940
LSD (0.05)	NS	NS	–	NS	NS	NS	NS
2005							
Aphthona spp.	192	648	–	700	152	456	2,148
Without *Aphthona* spp.	324	612	–	736	124	484	2,280
LSD (0.05)	NS	NS	–	NS	NS	NS	NS
Imazapic	156	688	–	848	168	412	2,272
Without imazapic	356	572	–	584	108	524	2,144
LSD (0.05)	NS	NS	–	256	NS	NS	NS
Warm-season grasses	196	604	–	860	140	508	2,308
Warm- plus cool-season grasses	220	716	–	756	136	152	2,180
Without grasses	352	764	–	540	140	544	2,140
LSD (0.05)	NS	NS	–	312	NS	NS	NS

[a] Yearly results were reported for the established treatments only. There was no interaction among treatments so main effects were averaged over all two- and three-way treatments.

[b] The "other grass" category was further subdivided into warm- and cool-season grasses after 2003.

[c] The total also includes shrubs and sedges, which were minor components of the biomass and not affected by the various treatments.

[d] *Aphthona* spp. flea beetles were released in June 2001.

[e] Imazapic at 105 g/ha was applied in September 2002.

[f] Native grasses were interseeded in May 2003.

Table 7. Change in herbage biomass over time as affected by *Aphthona* spp. flea beetles, imazapic, and interseeded native grasses at Walcott, ND.[a]

Year and treatment	Leafy spurge	*Poa* spp.	Other grass[b]	Forbs	Cool-season grasses	Warm-season grasses	Total[c]
	dry weight (kg/ha)						
2003							
Aphthona spp.[d]	450	717	2018	31	–	–	3,666
Without *Aphthona* spp.	726	457	1516	4	–	–	3,429
LSD (0.05)	NS	NS	NS	NS	–	–	NS
2004							
Aphthona spp.	360	64	–	64	460	180	1,128
Without *Aphthona* spp.	328	88	–	184	452	172	1,224
LSD (0.05)	NS	NS	–	NS	NS	NS	NS
Imazapic[e]	80	64	–	112	388	288	932
Without imazapic	612	84	–	136	524	64	1,420
LSD (0.05)	144	NS	–	NS	NS	172	100
2005							
Aphthona spp.	652	284	–	168	1,484	100	2,688
Without *Aphthona* spp.	796	316	–	296	988	192	2,588
LSD (0.05)	NS	NS	–	NS	NS	NS	NS
Imazapic	484	204	–	360	1,224	232	2,504
Without imazapic	946	396	–	104	1,244	60	2,808
LSD (0.05)	284	160	–	296	NS	132	NS
Warm-season grasses[f]	756	116	–	340	1,428	120	2,760
Warm- plus cool-season grasses	752	156	–	244	1120	296	2,568
Without grasses	664	628	–	108	1,156	20	2,576
LSD (0.05)	NS	196	–	NS	NS	160	NS
2006							
Aphthona spp.	690	222	–	126	1,678	119	2,835
Without *Aphthona* spp.	678	310	–	182	1,290	150	2,610
LSD (0.05)	NS	NS	–	NS	NS	NS	118
Imazapic	591	205	–	218	1,381	179	2,574
Without imazapic	777	327	–	89	1,587	91	2,871
LSD (0.05)	NS	NS	–	NS	NS	NS	NS
Warm-season grasses	597	188	–	196	1,691	167	2,839
Warm- plus cool-season grasses	684	201	–	128	1,363	224	2,600
Without grasses	771	410	–	137	1,398	13	2,729
LSD (0.05)	NS	219	–	NS	NS	146	NS

[a] Yearly results were reported for the established treatments only. There was no interaction among treatments so main effects were averaged over all two- and three-way treatments.

[b] The ''other grass'' category was further sub-divided into warm- and cool-season grasses after 2003.

[c] The total also includes shrubs and sedges which were minor components of the biomass and not affected by the various treatments.

[d] *Aphthona* spp. flea beetles were released in June 2002.

[e] Imazapic at 105 g/ha was applied in September 2003.

[f] Native grasses were interseeded in May 2004.

use is economically and environmentally prohibitive. Future research efforts to control leafy spurge in sandy soils should include evaluation of soil pathogens and the interaction of the pathogens with insect biological control agents.

Sources of Materials

[1] Scoil®, AGSCO, Inc., P.O. Box 13458, Grand Forks, ND 58208-3458.

[2] TeeJet Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

[3] Truax Company, Inc., 4300 Quebec Avenue North, New Hope, MN 55420.

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